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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/698,106	10/31/2003	Karla M. Robotti	10030204-1	8960

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EXAMINER	
BRADLEY, CHRISTINA	

ART UNIT	PAPER NUMBER
1654	

DATE MAILED: 05/02/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/698,106	ROBOTTI, KARLA M.	
	Examiner	Art Unit	
	Christina Bradley	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-21* is/are pending in the application.

4a) Of the above claim(s) *22-37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of Claims

Applicant's election without traverse of Group I, claims 1-21 in the reply filed on 4/12/2006 is acknowledged. Claims 22-37 are withdrawn from consideration.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1 and 4-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou *et al.* (*Nat. Biotech.*, **2001**, *19*, 375-378) in view of Kundu & Roy (*J. Lipid Res.*, **1979**, *20*, 825-833). Zhou *et al.* teach a method to purify phosphorylated peptides from a complex mixture of phosphorylated and unphosphorylated peptides and other molecules comprising the following steps: 1) protection of the peptide amino groups with *t*-butyl-dicarbonate (tBoc); 2) carbodiimide-catalyzed condensation of the peptide and an amine to form amide and phosphoramidate bonds at the carboxylate and phosphate bonds of the peptide, respectively; 3) regeneration of the phosphates by brief acid hydrolysis; 4) carbodiimide-catalyzed condensation of cystamine and the regenerated phosphate, and reduction of the internal disulfide of cystamine to generate a free sulfhydryl for every phosphate group of the peptide; 5) solid-phase capture of the

phosphopeptides by reaction of the sulfhydryl groups with iodoacetyl groups immobilized on glass beads; and 6) cleavage of phosphoramidate bonds with trifluoroacetic acid to regenerate the phosphates (see Figure 1). The method taught by Zhou *et* al. is similar to the method of claim 1 for the following reasons. Step 2 of Zhou *et al.* is equivalent to step a of claim 1 with the exception that Zhou *et al.* does not teach that the amine can be attached to a solid support, i.e. the first resin described in step a. Step 3 of Zhou *et al.* is equivalent to step b of claim 1. The TFA treatment used by Zhou *et al.* in step 3 achieves the same outcome as step b of claim 1: selective cleavage of the amine from the phosphate groups. Step 4 of Zhou *et al.* is equivalent to step c of claim 1. Here the cystamine of Zhou *et al.* functions as the capture ligand of claim 1, step c. Step 5 of Zhou *et al.* is equivalent to step d of claim 1. The reaction of the sulfhydryl groups with iodoacetyl groups attached to glass beads in step 5 of Zhou *et al.* accomplishes the same outcome as claim 1, step d: the separation of peptides bound to the capture ligand from peptides that are not bound to the capture ligand. Finally, step 1 of Zhou *et al.* meets the additional limitation described in claim 4: the protection of the peptide amine groups before the reaction with the first resin.

As stated above, Zhou *et al.* do not teach that the amine in step 2 is attached to a resin. Kundu & Ray teach a aminopropyl silica gel solid support which contains a free amino group available for reaction (see scheme 1). It would have been obvious to one of ordinary skill in the art to substitute the aminopropyl silica gel taught by Kundu & Roy for the amine in step 2 of Zhou *et al.* In doing so, one would meet all of the limitation of claims 1 and 5; the reaction in step 2 of Zhou *et al.* would involve a resin making it

equivalent to claim 1, step a, and that resin would comprise a primary amine as in claim 5. In addition, all of the limitations of claim 6 would be met because the TFA used in step 2 of Zhou *et* al. is a weak acid. The skilled artisan would have been motivated to make this combination given that the attachment of the amine in step 2 of Zhou to a solid support would permit the physical separation of the coupled peptides from the rest of the mixture and would permit extensive washing of the sample to further remove impurities without affecting the phosphopeptide (as performed in steps 5 and 6 in Zhou with the second resin). There would have been a reasonable expectation of success given that the chemical reaction required to couple the resin taught by Kundu & Roy to the peptide (the carbodiimide-catalyzed condensation of an amine and carboxylic acid) is routinely employed in protein chemistry and solid phase peptide synthesis (see Zhou *et al.*), and that the amino group in the resin taught by Kundu & Roy was shown to be coupled to another molecule. In addition, aminopropyl silica gel and functionally equivalent products are commercially available. Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 2, 3, 11 and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou *et al.* (*Nat. Biotech.*, **2001**, *19*, 375-378) as applied to claims 1 and 4-6 above in further view of Holmes (USPN 5,679,773). Step 6 of Zhou *et al.* meets the additional limitation described in claim 3: the cleavage of the bond between the capture ligand and the phosphorylated peptides following separation from the

unphosphorylated peptides in the preceding step. Zhou *et al.* do not teach that the amine of step 2 can be cleaved from the peptide prior to step 6 (i.e. that the first resin can be selectively cleaved before separating the peptides bound to the capture ligand as described in claim 2). Likewise, Zhou *et al.* do not teach that such cleavage can be photo-induced (claim 11) or that the first resin is photocleavable and has a plurality of groups represented by the structural formula in claim 12. Holmes teaches a photochemically-cleavable linking group identical to the formula in claim 12 (see column 14, compound 8) attached to a solid support such as a resin (see column 14, lines 40-50).

It would have been obvious to one of ordinary skill in the art to combine the method for phosphopeptide purification taught by Zhou *et al.* and the photochemically-cleavable linker resin taught by Holmes. The photochemically-cleavable linker resin would function as the first resin in claim 2, 3, 11 and 12. The skilled artisan would have been motivated to do so because Holmes teaches that the photochemically-cleavable linking group resin can be coupled via its amine to the carboxylic acid group of a peptide or amino acid (column 19, lines 10-19). In addition, the particular resin taught by Holmes is designed for highly-selective photoinduced cleavage (see abstract and Figure 2). By inducing such cleavage prior to step 6, unphosphorylated peptides can be isolated from the mixture. The light-induced cleavage would not interfere with the linkage between the second resin and the phosphopeptides. There would have been a reasonable expectation of success given that the chemical reaction required to couple the linker taught by Holmes to the peptide (the carbodiimide-catalyzed condensation of

an amine and carboxylic acid) is routinely employed in protein chemistry and solid phase peptide synthesis. Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 7-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou *et al.* and Holmes as applied to claims 2, 3, 11 and 12 above in further view of Fields *et al.* (*Proc. Natl. Acad. Sci.*, **1988**, *85*, 1384-1388). Zhou *et al.* do not teach that the amine of step 2 can be an amino acid coupled to a resin or that the amino acid can be isotopically labeled. Fields *et al.* teach the incorporation of an isotopically-labeled amino acid (Fmoc-[^{15}N]alanine) into a peptide by solid phase synthesis.

It would have been obvious to one of ordinary skill in the art to couple the Fmoc-[^{15}N]alanine taught by Fields *et al.* to the photocleavable-linker resin taught by Holmes, and to combine it with the method for phosphopeptide taught by Zhou *et al.* The Fmoc-[^{15}N]alanine-photocleavable-linker-resin, following deprotection of the amine, would be used in place of the amine in step 2 of Zhou *et al.* and would function as the first resin described in claims 7-10. The skilled artisan would have been motivated to make this modification given that the method would result in the incorporation of an isotope label useful for downstream mass spectrometry or NMR applications (see Zhou *et al.* and Fields *et al.*). There would have been a reasonable expectation of success given that the chemical reaction required to couple the linker taught by Holmes to the Fmoc-[^{15}N]alanine (the carbodiimide-catalyzed condensation of an amine and carboxylic acid) is routinely employed in protein chemistry and solid phase peptide synthesis (see Zhou

et al. and Fields *et al.*). Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 17-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou *et al.* and Holmes as applied to claims 2, 3, 10 and 11 above in further view of Oda *et al.* (*Nat. Biotech.*, **2001**, *19*, 379-382). Zhou *et al.* do not teach using a molecular recognition system to capture phosphopeptides. Oda *et al.* teach a method for enriching phosphorylated peptides from a complex mixture using the biotin-avidin interaction. The method of Oda *et al.* comprises converting the phosphate group in the peptides in several steps to biotin, and capturing the modified peptides with an avidin resin column (see Oda *et al.* Figure 1 and page 381, column 2).

It would have been obvious to one of ordinary skill in the art to replace steps 4-6 of Zhou *et al.* (including the modification from Holmes as described above) with the method taught by Oda *et al.* In doing so, the biotin would function as the capture ligand and the avidin resin would function as the affinity resin described in claims 18-21. The skilled artisan would have been motivated to make this modification given that Oda *et al.* use their method to enrich phosphopeptides from a complex mixture (the same purpose as the claimed method and the method in Zhou *et al.*), and that the biotin-avidin interaction has exceptionally high affinity and specificity. There would have been a reasonable expectation of success given that the methods taught by Oda *et al.* and Zhou *et al.* resulted in the purification of phosphopeptides from a complex mixture and that the use of the biotin-avidin molecular recognition system is ubiquitous in a variety of

applications. Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 13-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou *et al.*, Holmes and Fields *et al.* as applied to 2, 3 and 7-12 above in further view of Mercader *et al.* (*Anal. Biochem.*, **2002**, *308*, 269-277). Zhou *et al.* do not teach that the coupling and subsequent capture of the phosphate groups of steps 4-6 can be performed with an amino acid attached to a magnetic bead (i.e. that the capture ligand is a second resin and that the second resin is an amino acid attached to a magnetic bead). Mercader *et* al. teach the coupling of an aliphatic carboxylate group to Dynabeads M270 amine using a carbodiimide (see page 271). The Dynabeads are magnetic. Fields *et al.* teach Fmoc-protected amino acids and there use in solid phase peptide synthesis.

It would have been obvious to one of ordinary skill in the art to couple an Fmoc-amino acid (such as Fmoc-tryptophan) of Fields *et al.* to the Dynabeads by the method in Mercader *et al.* and, following deprotection of the amine, use it to react with the phosphate groups following step 3 of Zhou *et al.* In doing so, the amino acid would function as the capture ligand. Because it is coupled to a magnetic bead, the capture ligand would also be a second resin. All limitations of claims 13-16 would be met. One would have been motivated to make this modification because magnetic beads permit easy separation of a captured compound from a mixture as suggested by Mercader *et al.* There would have been a reasonable expectation of success given that given that

the chemical reaction required to couple the amino acid and the phosphate groups of the phosphopeptides is the same as that used successfully in step 2 of Zhou *et al.* Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christina Bradley whose telephone number is (571) 272-9044. The examiner can normally be reached on Monday through Friday, 8:30 A.M. to 5:00 P.M..

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Bruce Campell can be reached on (571) 272-0974. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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